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## (54) Transgenic tomato plants with altered polygalacturonase isoforms.

(57) A method of creating a transgenic tomato containing a lowered level of polygalacturonase isoform 1 is disclosed. This method begins with the step of isolating a DNA sequence encoding at least a portion of the polygalacturonase beta-subunit. This portion is sufficient to hybridize effectively to the mRNA of the polygalacturonase beta-subunit in vivo. A genetic construction is created from the cDNA clone in which the cDNA clone is positioned so that the antisense version of the polygalacturonase beta-subunit message may be produced from the clone. A tomato cell is transformed with the construction, whereby the tomato cell produces in the fruit a lowered level of PG beta-subunit and, hence, lowered levels of polygalacturonase isoform 1.

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## Field Of The Invention

The present invention relates generally to transgenic plants. Specifically, the present invention relates to a transgenic tomato plant with altered levels of isoforms of the enzyme polygalacturonase.

## Background Of The Invention

### Polygalacturonase

In recent years *Lycopersicon esculentum*, the cultivated tomato, has become a popular system for studying fruit ripening. Tomato fruit ripening is characterized by a series of coordinated biochemical and physiological changes within the various subcellular compartments of the fruit tissue. These changes collectively contribute to the overall quality of the ripe fruit. The most obvious of the changes are alterations in fruit color, flavor, texture and resistance to certain pathogens.

One biochemical change in ripening fruit is the depolymerisation and solubilization of cell wall polyuronides by the ripening-induced cell wall degrading enzyme, polygalacturonase (PG). PG activity increases dramatically during the ripening of many fruits, including tomato, and is the primary enzymic activity responsible for cell wall polyuronide degradation during fruit ripening. Reviewed in Giovannoni, et al, 1991 Ann. Rev. Hortic Sci: 67-103.

PG activity isolated from ripe tomato fruit is due to the presence of three structurally and immunologically-related isoforms of PG. These isoforms are termed PG1, PG2A and PG2B. (Ali, et al. Aust. J. Plant Physiol. 9:171, 1982). The PG2A and PG2B isoforms (45 and 46 kDa, respectively) appear well after the onset of ripening and are each composed of a single catalytic PG polypeptide differing only in degree of glycosylation. Because of the physical and biochemical similarity of PG2A and PG2B, the two isoforms shall be treated herein as a single isoform activity (the PG2 activity).

The PG1 isoform (approximately 100 kDa) is the first isoform to appear, at the onset of ripening, and is a heterodimer composed of the single catalytic PG2 polypeptide (either PG2A or PG2B) tightly associated with an ancillary cell wall glycoprotein, the PG beta-subunit. The formation of PG1 by association of the PG2 polypeptide with the PG beta-subunit protein alters both the biochemical and enzymic properties of the associated catalytic PG2 protein. The isoelectric point and pH optimum of PG1 are both a full unit lower than those of PG2. PG1 is more thermo-stable than PG2. PG1 retains complete activity after heating for 5 minutes at 65°C, a treatment that completely inactivates PG2.

In recent years, cDNA clones for the catalytic PG2 polypeptide have been identified and used to examine in detail the regulation of PG gene expression in wild-type and mutant tomato fruit (DellaPenna, et al, Proc. Natl. Acad. Sci. USA 83:6420 (1986). Analysis of PG2 genomic and cDNA clones has revealed that the catalytic PG polypeptide is encoded by a single gene which is transcriptionally activated at the onset of wild-type fruit ripening (DellaPenna et al, Plant Physiol. 90:1372 (1989). PG2 mRNA is synthesized de novo during the ripening of wild-type fruit and accumulates to high levels, accounting for greater than 1% of the mRNA mass. Ripening-impaired mutants of tomato, which are inhibited in many ripening processes including PG2 expression, have greatly reduced levels of PG2 mRNA. The severe reduction in steady-state PG mRNA levels in the mutant genotypes is due to greatly reduced transcriptional activity of the PG gene (DellaPenna, et al, 1989, supra).

The PG beta-subunit has also been studied. The levels of PG beta-subunit increase approximately 4-fold during fruit ripening (Pressey, R., Eur. J. Biochem. 144:217-221 (1984)) and apparently determine the amount of PG1 produced during tomato ripening. Therefore, as PG beta-subunit levels are depleted (by formation of PG1), the timing of appearance of the PG2 isoform is also controlled.

While it is clear from in vitro studies that PG1 and PG2 differ in their biochemical properties, the physiological significance of the isoforms and the role of the PG beta-subunit protein remains uncertain. From a physiological point of view, it seems likely that a cell wall enzyme like PG might be localized or its activity restricted to specific regions of the cell wall by association with an adhesion or localizing factor, such as the PG beta-subunit protein. Recent results in transgenic systems have also suggested that PG1 may be the physiologically active isoform in vivo with regard to pectin degradation, presumably due to its association with the PG beta-subunit protein.

Pectolytic enzymes, such as PG, may have a role in plant pathogen interactions. Pathogen-derived pectolytic enzymes are thought to be important components of the mechanism by which pathogens penetrate and colonize plant tissues. Preliminary results from recently completed experiments have suggested that PG induction in transgenic mutant fruit increases colonization of the fruit by *Alternaria alternata*, a common late-season pathogen of wild-type tomato fruit to which mutant fruit are normally resistant. The apparent conferral

of pathogen sensitivity to mutant fruit by the specific induction of PG expression suggests that increasing PG activity during fruit ripening may play an important role in altering the susceptibility of the fruit to pathogens.

In addition, antisense inhibition of PG expression in wild-type tomato has been correlated with a decrease in "fieldrot" during later stages of tomato ripening in the field. (Kramer, et al, 1990, Horticultural Biotechnology, pp 347-355, Wiley-Liss Inc.). Although the mechanism of PG associated alterations in pathogen susceptibility is not known, these results strongly suggest a role for PG in postharvest pathogenesis.

Tomato Processing

An important determinant of many processed tomato fruit products, including sauce, paste and catsup, is the viscosity (i.e. thickness) of the final product. One of the primary determinants of high viscosity is the presence of large, unmodified pectin molecules. Pectin is a naturally occurring plant cell wall carbohydrate polymer that is composed primarily of polygalacturonic acid residues. Maintenance of pectin integrity during tomato processing is an extremely important part of the commercial process.

An important factor in loss of pectin integrity (decrease in the polymer size and subsequent loss of viscosity) during commercial processing of tomatoes is enzymatic degradation of pectin by PG. Although some modification of pectins by PG occurs naturally during the ripening process (DellaPenna, et al, Plant Physiology 94:1882-86, (1990)), by far the most dramatic and commercially damaging action of PG on pectins and, hence, viscosity occurs when the tomato fruit is homogenized for processing. The PG enzyme present in the fruit has the potential to act in an uncontrolled fashion in homogenized fruit tissues and can rapidly degrade pectin polymers.

A rapid, high-temperature heat treatment is used in commercial tomato processing to destroy PG enzyme activity and thereby maintain a higher viscosity in the final product. This treatment often comprises a process known as "hot break" and is performed by the rapid heating of the tomato product to near boiling point, to inactivate the PG enzyme as rapidly as possible. The annual cost associated with the input of large amounts of energy to bring millions of tons of tomatoes to the temperature needed to rapidly inactivate PG represents a significant cost to tomato processing industries. Annual tomato production in the US is approximately 6 million metric tons representing approximately 10% of worldwide production (1980 figures).

It follows that any process that would allow less energy to be used to inactivate PG in tomato products would result in substantial savings to the industry. A process that would decrease the thermal stability of the PG isoforms would therefore decrease the minimum temperature needed to heat-inactivate PG during processing. All commercially useful, non-genetically engineered tomato varieties currently on the market contain both PG1 and PG2 isoforms. Generally, 10-30% of total PG activity is PG1 in a ripe fruit. One way to decrease the thermal stability of the PG isoforms would be to inactivate or lessen the amount of PG1, the more thermostable PG isoform.

Antisense RNA

It has been found in both procaryotes and eukaryotes, that the production of specific endogenous proteins can be inhibited by use of an antisense RNA. An "antisense RNA" is a complementary version of a naturally occurring or endogenously produced RNA. Because of its complementary sequence, the antisense RNA will hybridize to the mRNA of the protein sought to be inhibited under physiological conditions. This hybridization prevents translation and, therefore, protein production. The duplex RNA complex thus formed is eventually degraded by appropriate cellular mechanisms, without resulting in expression of a protein. An antisense RNA can conveniently be formed for a known protein coding region by reversing the orientation of the protein coding region so that the end that is normally transcribed last is now transcribed first.

Investigators have inhibited production of the catalytic PG2 polypeptide by antisense RNA technology and have shown a greater than 92% reduction of total PG activity relative to wild-type activity levels (Kramer, et al., Horticultural Biotechnology, 1990, pp. 347-355, Wiley-Liss, Inc.). This reduction had significant effects on processed tomato product viscosity when the product was subjected to the normal "hot break methods. These investigators did not, however, determine which PG isoforms were produced in the transgenic fruit. One would expect that the PG2 polypeptide levels were greatly reduced and that all of the PG activity formed would be in the PG1 (heat-stable) isoform due to the presence of existing beta-subunit protein, which would not have been affected by antisense inhibition of PG2 protein.

Inhibiting the level of PG2 expression is not the equivalent of lowered levels of PG1. The reduction of catalytic PG2 polypeptide levels results in the lowering of total PG activity levels and PG2 protein levels, without affecting the formation of the heat stable PG1 isoform directly except by reducing the amount of PG2 available to form PG1. Because 100% inhibition of PG2 production has not been reported, any residual PG2 produced

10,000 g for 20 minutes, resuspended in one half volume of cold $H_2O$ at pH 3.0 and repelleted. The cell debris pellet was resuspended in cold buffer contained 50 mM sodium acetate, 1.25 M NaCl (pH 6.0) and stirred for at least one hour at 4°C. The extract was centrifuged at 10,000 g for 20 minutes and proteins in the supernatant were precipitated by the addition of ammonium sulfate to 70% saturation. After centrifugation, the resulting protein pellet was resuspended in 0.125 M sodium acetate (pH 6.0) and dialyzed extensively against the same buffer. The dialyzed extract was then clarified by centrifugation and applied to a CM-Sepharose column equilibrated with 0.125 M sodium acetate (pH 6.0). Bound proteins were eluted by a two step gradient of 0.45 M sodium acetate (pH 6.0) and 1.0 M sodium acetate (pH 6.0). PG2 A and B eluted with 0.45 M sodium acetate while PG1 eluted with 1.0 M sodium acetate.

The 1.0 M sodium acetate eluent was concentrated by ultrafiltration, dialyzed against Concanavalin A (Con-A) buffer [500 mM NaCl, 50 mM sodium acetate, 1 mM calcium acetate, 1 mM manganese sulfate (pH6.0)] and further purified by Con-A chromatography as previously described (DellaPenna, 1986 supra). PG1-containing fractions were concentrated by ultrafiltration, dialyzed against 50 mM phosphate, 200 mM NaCl, 0.1 mM DDT (pH6.0) and further purified by Mono S FPLC chromatography, Pogson et al, Aust. J. Plant Phys. 18:65-79 (1991).

The subunits of PG1 were separated and isolated as described (Pogson et al., 1991 supra). PG1 purification and separation of PG2 and the beta-subunit protein were followed by SDS-PAGE. Electrophoretic blotting, and detection methods for the catalytic PG polypeptide were performed as described previously (DellaPenna et al., 1986, supra). PG1 and PG2 levels during extraction and purification were determined by heat inactivation (Tucker et al., Eur. J. Biochem. 115:87-90, 1981) and activity staining of protein extracts separated by non-denaturing PAGE (DellaPenna, 1987 supra).

N-terminal sequence analysis of the purified beta-subunit was performed with a Beckman 890M gas phase sequenator. Internal beta-subunit proteolytic fragments were generated by digestion with Lys-C and Glu-C endoproteases following instructions supplied by the manufacturer (Promega, Madison, Wisconsin). The resulting proteolytic fragments were resolved by SDS-PAGE, blotted to PVDF membranes and directly sequenced.

Two internal peptide fragments were of interest. The amino acid sequence of the Lys-C peptide was: $NH_2$-Asn-Gly-Asn-Gly-Ala-Asn-Gly-Gln-[?]-Val (SEQ ID NO: 1). The amino acid sequence of the Glu-C peptide was: $NH_2$-Ala-Asn-Ala-Gly-Asp-Gln-Tyr (SEQ ID NO: 2). The underlined portion of these sequences indicates the sequence from which a nucleotide primer was constructed. These nucleotide primers are presented at SEQ ID NO: 3 (for the Lys-C primer) and 4 (for the Glu-C primer).

These degenerate oligonucleotides were used for library screening and PCR-based MOPAC generation of cDNA probes. One microgram of poly (A)+RNA from immature green, mature green, turning and fully ripe tomato pericarp tissues was used in PCR-based MOPAC reactions. A Not I primer-adaptor (Promega, Madison, WI) was used as a primer for first strand cDNA synthesis. Subsequent PCR amplification cycles utilized a Not I adaptor as the 3' primer and a degenerate 5' primer (SEQ ID NO: 4), derived from the Glu-C beta-subunit protease fragment. Amplified products were electrophoresed, blotted to nylon membranes and probed with a second degenerate oligonucleotide (SEQ ID NO: 3) derived from the Lys-C beta-subunit protease fragment. The Lys-C primer recognized a 1.3 kb product generated in the MOPAC reactions. This 1.3 kb product was recovered and amplified by PCR using the Lys-C primer (5'-end) and the Not I adaptor (3'-end). This second 1.25 kb MOPAC-derived PCR product was used in conjunction with degenerate oligonucleotides for library screening and Northern analysis. Oligonucleotide 5'-end labelling and random primer DNA labelling were performed following the manufacturer's protocol (BRL, Gaithersburg, MD).

The cDNA library contained $1.0 \times 10^7$ individual recombinants before amplification. For primary screening, replica nitrocellose filters (25,000 pfu/plate) were probed with the degenerate 17-mer Lys-C oligonucleotide described above. Prehybridization was carried out for 4 hours at 37°C in a solution of 6 X SSC; 1 X Denhardt's solution; 0.5% SDS; 0.05% sodium pyrophosphate; 100 ug/ml denatured salmon sperm DNA. Hybridization was carried out overnight at 37°C in 6 X SSC; 1 X Denhardt's solution; 20 ug/ml tRNA; 0.05% sodium pyrophosphate. Following hybridization, the filters were washed twice for 5 minutes at room temperature and twice at 37°C for 30 minutes in 5 X SSC; 0.05% sodium pyrophosphate. A final wash was performed in 5 X SSC; 0.05% sodium pyrophosphate at 40°C for 10 minutes. The filters were exposed overnight with intensifying screens at -80°C.

Further rounds of screening were performed at low density using the PCR-generated MOPAC cDNA fragment (described above), the Glu-C oligonucleotide and a degenerate N-terminal oligonucleotide [5'-AT(AG) TCX CC(AG) CT(GA) TG(CT) TT(CT) TC (SEQ ID NO: 5)] derived from the N-terminal protein sequence. Hybridization conditions for these oligonucleotides were as described above. Hybridization conditions used with the MOPAC-generated cDNA fragments were as described by Sambook et al 1989, (supra). Following plaque purification, plasmids were rescued by in vivo excision, following the manufacture's protocol (Stratagene). Dou-

ble-stranded DNA sequencing was performed.

All the beta-subunit clones we obtained have the restriction pattern illustrated in Fig. 1. We have sequenced two beta-subunit cDNA clones and fragments of 11 others. SEQ ID NO: 6 is the consensus DNA sequence we obtained from the clones. A poly-(A) tail of 35 residues was found at the end of the cDNA but has been removed from SEQ ID NO: 6.

3. Antisense Expression of the Beta-Subunit cDNA

Expression of the beta-subunit protein will be modified by introducing Cauliflower Mosaic Virus (CaMV) 35S promoter driven chimeric genes containing a full-length beta-subunit cDNA in the antisense orientation into various tomato genotypes. This highly expressed constitutive promoter is widely available. Other promoters may also be utilized. The constitutive CaMV 35S promoter will be initially used for the proposed experiments because this promoter has been shown to promote high levels of protein production in most plant organs, including tomato fruit. Kramer, et al., supra.

The feasibility of reducing the expression of tomato fruit genes by antisense RNA technology has been demonstrated by Kramer, et al. (supra). A similar strategy will be employed to inhibit expression of the beta-subunit protein in transgenic tomato plants. Antisense repression of beta-subunit protein production will greatly decrease the amount of beta-subunit protein available for the formation of PG1, thereby resulting in accumulation of only the PG2 isoforms.

First, an antisense DNA construction will be created. At a minimum, this DNA construction must contain a promoter effective to promote transcription in tomato plants, an antisense version of a cDNA clone encoding PG beta-subunit, and a sequence effective to terminate transcription. Via standard molecular biological methods, the CaMV35S promoter sequence will be attached to the beta-subunit cDNA insert. The cDNA insert will be in the antisense orientation. This orientation will be accomplished by attaching the 3' end of the cDNA insert to the promoter. Preferably, the poly-(A) tail will be removed for the antisense construction with the enzyme NruI which will leave a DNA fragment of approximately 1800 bp for antisense construction (approximately 400 bp of 3' sequence plus the poly-A tail will be deleted by this method). Therefore, the antisense RNA strand will be the transcription product.

A suitable termination sequence, such as the nopaline synthase 3' terminator, will be placed downstream from the cDNA insert.

The DNA construction will be placed in an appropriate vector for plant transformation. For Agrobacterium-mediated transformation, the promoter/cDNA/terminator construction will preferably be placed in a Ti-based plasmid, such as pBI121, a standard binary vector.

In general, transformation will preferably be done with two standard Aarobacterium binary vectors: pBI121 (sold by Clontech Laboratories, Palo Alto CA) and pGA643 (developed by G. An at Washington State University). pBI121 contains a CAMV promoter and GUS reporter gene. The GUS coding sequence will be removed by digesting with SstI and SmaI (blunt end). The beta-subunit DNA fragment to be used will be produced by digesting with SstI (sticky end) and NruI (blunt end). The sticky/blunt ends will allow for directional cloning into pBI121 in the antisense orientation. Standard methods for cutting, ligating and E. coli transformation will be used.

For plant transformation we will follow, in general, the methods of McCormick (1986, Plant Cell Reporter 5:81-84) and Plant Tissue Culture Manual B6:1-9 (1991) Kluwer Academic Publishers. This later reference compiles/compares various procedures for Agrobacterium-mediated transformation of tomato.

The level of beta-subunit, individual PG isozymes and overall heat stability of total PG activity as a function of time and temperature in the tomato will then be analyzed. Fruit from transgenic antisense tomatoes can be processed and heat-treated to assess levels of PG1 activity. If as the tomato pulp is heated to between 65°C and 90°C, and PG activity is halted more rapidly than wild-type fruit, the effort would have been successful.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: The Arizona Board of Regents on Behalf of The University o
(B) STREET: 1430 East Fort Lowell Road, Suite 200
(C) CITY: Tuscon
(D) STATE: Arizona
(E) COUNTRY: USA
(F) POSTAL CODE (ZIP): 85719

(ii) TITLE OF INVENTION: Transgenic Tomato Plant with Lowered Level of Polygalacturonase Isoform 1

(iii) NUMBER OF SEQUENCES: 6

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: EP 93303533.9
(B) FILING DATE: 07-MAY-1993

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Lycopersicon esculentum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Asn Gly Asn Gly Ala Asn Gly Gln Xaa Val
1 5 10

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: internal

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Lycopersicon esculentum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Ala Asn Ala Gly Asp Gln Tyr
1 5

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GGNAAYGGNG CNAAYGG 17

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

AAYGCNGGNG AYCARTA 17

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: Oligonucleotide

(iii) HYPOTHETICAL: YES

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

ATRTCNCCRC TRTGYTTYTC 20

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2192 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: Lycopersicon esculentum

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

TCTCTCTCTT CATCTCTGTT TCACACCAAA GAAATGCACA CTAAAATTCA TCTTCCTCCC 60
TGCATCTTAC TTCTTCTTCT GTTCTCACTA CCATCTTTCA ATGTTGTTGT AGGTGGAGAT 120
GGTGAATCTG GTAACCCATT TACACCCAAA GGTTATCTGA TTAGGTACTG GAAGAAACAA 180
ATCTCAAATG ACTTACCAAA GCCATGGTTT CTTCTGAACA AGGCATCTCC ATTGAATGCT 240
GCACAATATG CAACTTACAC TAAACTTGTT GCTGATCAAA ATGCACTCAC CACACAGCTC 300
CATACCTTTT GCTCTTCAGC AAATCTCATG TGTGCACCAG ATCTGTCACC AAGTCTTGAA 360
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GGCGGTGAAG CGAATTTCAC CAACTATGGT CAATCATTCA ATGAAGGTAC TGATGTATTC 1080
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AGTATGAATG GCTATCAATT TACACTATTT GTTATGTAAT CATTTTATTG TTGACTCATA 2160
TTTGAGCAAG GTAATGTAGT TATTGCCAGA TG                                2192
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## Claims

1. A method of creating a transgenic tomato containing a lowered level of polygalacturonase isoform 1, comprising the steps of
   a) isolating a DNA sequence encoding at least a portion of the polygalacturonase beta-subunit, wherein the portion is sufficient to hybridize effectively to an mRNA for the polygalacturonase beta-subunit;
   b) creating a genetic construction from the DNA sequence, wherein the DNA sequence is positioned so that an antisense polygalacturonase beta-subunit RNA is produced from the construction; and
   c) transforming a tomato cell with the genetic construction, whereby the tomato cell produces a lowered level of polygalacturonase isoform 1.

2. The method of claim 1 wherein the transforming step is performed by Agrobacterium-mediated transformation.

3. The method of claim 1 or 2 wherein the cDNA clone has substantial homology to SEQ ID NO: 6.

4. A genetic construction comprised of:
   a) a promoter capable of expressing a downstream coding sequence in a tomato plant;
   b) a sequence encoding an RNA of at least fifteen nucleotides complementary to the mRNA of tomato polygalacturonase beta-subunit; and
   c) a 3' termination sequence.

5. The construction of claim 4 wherein the sequence encoding the polygalacturonase beta-subunit is substantially homologous to SEQ ID NO: 6.

6. A bacteria containing the construction of claim 4 or 5.

7. A transgenic tomato fruit, wherein said tomato has lowered levels of the polygalacturonase isoform PG1.

8. A transgenic tomato fruit, wherein said tomato has lowered levels of polygalacturonase beta-subunit.

9. A transgenic tomato containing the construction of claim 4 or 5

10. A transgenic tomato plant comprising in its genome a foreign genetic construction comprising a promoter effective in tomato, a coding region encoding an RNA of at least 15 nucleotides complementary to the mRNA of the tomato polygalacturonase beta-subunit gene, and a transcriptional terminator.

11. Seed of the tomato plant of claim 10.

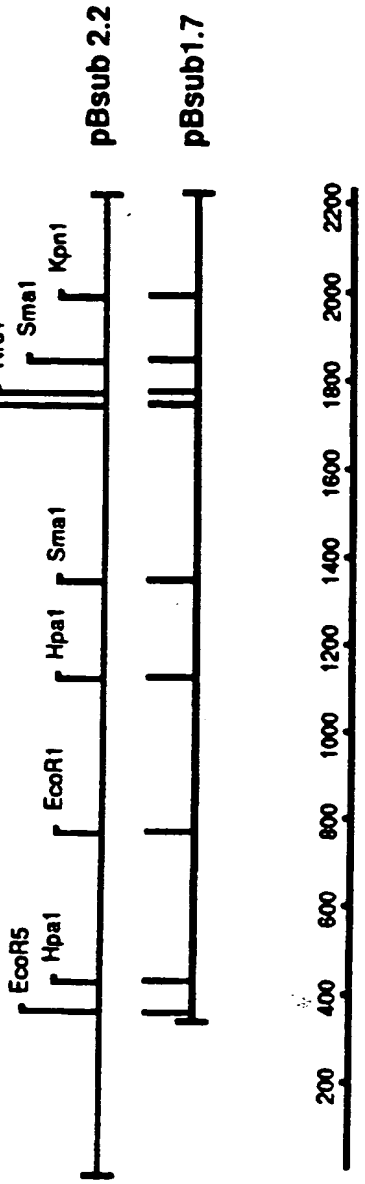
pBsub 2.2
pBsub1.7
EcoR5
Hpa1
EcoR1
Hpa1
Sma1
EcoR1
Nru1
Sma1
Kpn1
200
400
600
800
1000
1200
1400
1600
1800
2000
2200

FIG. 1

European Patent Office

# EUROPEAN SEARCH REPORT

Application Number

EP 93 30 3533



## DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int. Cl.5) |
|---|---|---|---|
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The present search report has been drawn up for all claims

| Place of search | Date of completion of the search | Examiner |
|---|---|---|
| MUNICH | 08 SEPTEMBER 1993 | YEATS S. |

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# EUROPEAN SEARCH REPORT

Application Number

EP 93 30 3533



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| Category | Citation of document with indication, where appropriate, of relevant passages | Relevant to claim | CLASSIFICATION OF THE APPLICATION (Int. Cl.5) |
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